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THE UNIVERSITY OF ALBERTA

COMPARISONS OF UREA RECYCLING IN CARIBOU, CATTLE, AND SHEEP

BY

© RICHARD ALLEN WALES

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF MASTER OF SCIENCE

DEPARTMENT OF ANIMAL SCIENCE

EDMONTON, ALBERTA

SPRING, 1972

Thesis
1972
151

THE UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled "Comparisons of Urea Recycling in Caribou, Cattle, and Sheep" submitted by Richard Allen Wales, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

ABSTRACT

The technique of Ford (1969) entailing use of ^{14}C-urea was employed to estimate the proportion and quantity of plasma urea transferred to the digestive tracts of two mature, captive caribou bulls and two yearling beef bulls maintained on diets of low protein contents. Trials were conducted on caribou (Rangifer tarandus) during both the summer growth and winter dormancy phases of the growth cycle. Data on both cattle and caribou were compared to results obtained by Ford (1969) using sheep under similar conditions of management.

The beef bulls had substantially lower plasma urea concentrations than either the sheep or the caribou consuming similar amounts of dietary nitrogen per unit of metabolic body size. The dietary nitrogen intake of the winter caribou was reduced to two thirds of the intake of the summer caribou per unit of metabolic body size, yet the plasma urea levels of the winter caribou were slightly higher. The beef bulls and the summer caribou both recycled approximately 47% of the injected label, compared to an average of approximately 41% recycled by the sheep. Thus, the summer caribou and the beef bulls tended to recycle a greater proportion of the injected label than did the sheep, but the difference was not statistically significant.

The winter caribou recycled about 60% of the injected label, which was a significantly greater portion than was recycled by the beef bulls and the summer caribou ($P < 0.05$) and by the sheep ($P < 0.01$). The quantity of urea recycled per unit of metabolic body size, from greatest to least, was in the order of winter caribou, sheep, summer caribou and beef bulls.

The present findings generally supported previous work indicating that the quantity of urea recycled is proportional to the plasma urea concentration. This conclusion was based on the fact that in the cases where the proportion of label recycled was fairly similar in terms of group averages, the quantity of urea recycled depended on the plasma urea concentration. However, the greater quantity of urea recycled by the winter caribou occurred without a concomitant increase in the plasma urea concentration. Rather, the proportion of label recycled increased, which was considered indicative of a shift in the disposition of plasma urea.

Mechanisms which might be involved in the apparent increase of urea recycling noted in _Rangifer tarandus_ during the winter dormancy period were discussed. Suggestions were made as to areas where further research efforts might profitably be directed.

ACKNOWLEDGEMENTS

The author wishes to thank Dr. L.W. McElroy, Chairman, Department of Animal Science, for use of the facilities of the Department. Sincere thanks must also go to Mr. J.A. Francis for taking care of the beef bulls, and for his competent assistance in collecting samples.

Without the complete cooperation of Dr. E.H. McEwan, Research Scientist, Canadian Wildlife Service, who unselfishly shared animals and facilities, this study could not have been possible. I am also grateful for his guidance and assistance in working with the caribou bulls used in these experiments.

Special thanks are extended to Dr. L.P. Milligan, Associate Professor of Animal Biochemistry, for his supervision, guidance, and constructive criticism. Above all I appreciate his patience and understanding; his ability to offer assistance and encouragement without oppression.

Acknowledgement is given to the Alberta Agricultural Research Trust and to the National Research Council of Canada for providing financial support for this project.

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INTRODUCTION

It is now well established that urea recycled from the blood to the rumen, both directly across the rumen wall and via saliva, can contribute significantly to the nitrogen economy of ruminant animals. In trials conducted on sheep by Ford (1969), recycled urea provided nearly one third as much nitrogen to the digestive tract as was available from the ration. Numerous other groups working with domestic species of ruminants have demonstrated an increase in urea recycling under conditions of low dietary nitrogen intake, particularly when an adequate source of carbohydrate was available (Houpt, 1959; Schmidt-Nielsen and Osaki, 1958; Thornton, 1970c). Studies by Schmidt-Nielsen (1958) indicated that camels are also able to increase utilization of endogenous urea under conditions of low protein intake, or in circumstances where low protein intake is combined with growth of the animal. The ability to recycle urea to enhance rumen microbial protein synthesis may well constitute the margin of survival for free-ranging ruminants living in areas of unfavorable nutritional conditions.

The present studies were undertaken to estimate the extent of urea recycling in captive caribou during both the summer growth phase and the winter dormancy period. It was reasoned that such a study might shed light on the question of why caribou are apparently better adapted than domestic stock to the harsh foraging conditions of the arctic tundra. In addition, it was desired to ascertain if real, quantitative differences in recycling of plasma urea can exist in different species of ruminants, or within an individual animal at different points in time. All trials, including two comparative

trials on yearling beef bulls, were conducted utilizing the ^{14}C tracer technique developed by Ford (1969) at the University of Alberta.

REVIEW OF LITERATURE

A. Kidney Urea Retention

Until quite recently, blood urea was considered solely as a waste product of nitrogen metabolism, to be totally excreted from the body (Addis et al., 1947). The work of Schmidt-Nielsen et al. (1957) suggested that ruminants, and especially young, growing individuals, under conditions of low protein intake, might differ from other mammals in their ability to withhold urea from urinary excretion (Schmidt-Nielsen et al., 1957; Schmidt-Nielsen, 1958; Schmidt-Nielsen and Osaki, 1958).

Schmidt-Nielsen (1958) concluded that the regulation of urea excretion is at the tubular level of the kidneys. She postulated a counter-current multiplier mechanism operating at the kidney tubular level, the activity of which could be regulated in order to concentrate urea in the urine. Many subsequent interspecies comparisons by a number of groups (Atherton et al., 1967; Clapp, 1966; Gans, 1966; Lassiter et al., 1966; Schmidt-Nielsen, 1962) have resulted in a currently accepted concept of this mechanism as presented by Morgan and Berliner (1968).

Operating in conjunction with control exerted at the counter-current multiplier, urea reabsorption from the collecting ducts is considered to be an important means of reducing urea excretion; it has been suggested that antidiuretic hormone may mediate the control of this urea reabsorption (Bowman and Foulkes, 1970). However, while some authors suggest a permissive effect of antidiuretic hormone on passive diffusion (Berliner and Bennett, 1967; Bowman and Foulkes,

1970; Levinsky and Berliner, 1959; Ullrich et al., 1967), other workers postulate an effect on a transport system involved in actively reabsorbing urea from the collecting ducts (Lassiter et al., 1966; Truniger and Schmidt-Nielsen, 1964). The reported increase in urea reabsorption from collecting ducts under conditions of protein depletion (Clapp, 1966) appears likely to be linked to some such mechanism.

The effects of plasma urea concentration and urine flow rate on the renal regulation of urea excretion are important to any consideration of urea recycling, but have yet to be established conclusively. Recent studies (Cocimano and Leng, 1967; Thornton, 1970a, b, d) tend to contradict earlier work (Gans, 1966; Lassiter et al, 1966; Schmidt-Nielsen and Osaki, 1958; Schmidt-Nielsen et al., 1958) in suggesting that urinary urea excretion is positively related to plasma urea concentration.

B. Recycling of Urea to the Rumen

The first person to suggest that direct transfer of blood urea nitrogen to the rumen might occur to any significant extent was Houpt (1959), although previous workers had indicated retention of urinary urea under certain conditions (McDonald, 1948; Schmidt-Nielsen, 1957). Saliva was generally considered to be the means of transport from the blood to the rumen. In an attempt to quantitate modes of urea transport from blood to rumen, Houpt (1959) measured the excess over basal urea in the urine following an intravenously injected urea load. The amount of urea retained by the body, as represented by the difference between the injected load and the excess urea over basal in the urine, was assumed to have passed into the rumen.

Subsequent acute experiments were designed to estimate the proportion of blood urea transferred directly across the rumen wall as opposed to salivary transfer. With salivary supply to the rumen blocked, measurement of accumulated ammonia in the rumen of a sheep with contents replaced with saline, together with measurements of ammonia reabsorption from the rumen, gave an estimated rate of direct urea recycling to the rumen. Since Houpt's first work in 1959, a great deal of research by various groups has conclusively established that direct transfer of blood urea to the digestive tract does occur to a significant extent.

However, the mechanism(s) by which this transfer occurs is still very much a controversy. Three main camps of thought have developed. Gartner, Decker and Hill (1961) proposed active transport of urea into the rumen, on the basis of their results suggesting that increased blood urea levels did not affect the rate of transport of urea into the rumen. Conversely, Engelhardt and Nickel (1965) considered urea passage into the rumen of sheep and goats to be by means of passive diffusion. Their conclusions have subsequently been supported by a number of groups (Cocimano and Leng, 1967; Houpt and Houpt, 1968; Juhasz, 1965).

Cocimano and Leng (1967), using a variety of rations with differing protein contents to vary blood urea levels, suggested a linear relationship between plasma urea concentration and urea entry rate into the body pool. In support of this linear relationship, Houpt and Houpt (1968) proposed that urea nitrogen is transferred by passive diffusion across the rumen wall according to the concentration gradient. They concluded that while urea per se moves through the wall by diffusion, under normal rumen conditions blood urea is hydrolyzed

by urease of bacterial origin before all epithelial diffusion barriers are crossed. The ammonia produced, being a smaller molecule than urea, was proposed to diffuse more rapidly through the remaining epithelial layers, with the result that transfer of urea nitrogen from blood into the rumen is enhanced by the association of bacterial urease with the rumen wall.

Ford (1969) studied urea recycling in sheep and proposed that simple diffusion was the means of transfer of urea from plasma into the rumen. In his study, recycled urea was linearly related to plasma urea concentration only within the normal physiological range of plasma urea concentrations. Above a threshold of about 45 mg urea/100 ml plasma, recycled urea was maximal and no longer linearly related to plasma urea concentration. Ford (1969) proposed that at elevated plasma urea concentrations, urea forms loosely bound, reversible complexes with plasma proteins such that the additional urea neither effectively contributes to the diffusion gradient nor is filtered in the kidneys.

A third major hypothesis suggests that rumen ammonia concentration is a central factor in the control of urea transfer to the rumen. Results obtained from trials on starved sheep subjected to intravenous urea loads gave Varady et al. (1967) reason to suggest that the amount of urea nitrogen transferred from blood to rumen depends on the blood urea concentration only as long as the rumen ammonia concentration remains sufficiently low. The rumen ammonia concentration at which inhibition of urea transfer to the rumen began was not determined in their experiments, but at a rumen ammonia concentration of 12 m mol/l and under conditions of intravenous urea loading, urea passage was so

low that the rumen ammonia concentration remained practically unchanged. Weston and Hogan (1967) also suggested a maximal rumen ammonia concentration which was not increased by further elevation of blood urea levels. Working with sheep consuming a chopped hay diet, they indicated a maximal transfer of urea to the rumen at rumen ammonia concentrations up to 8-10 mg nitrogen per 100 ml rumen fluid. Under the conditions of their experiment, this rumen ammonia concentration was achieved at a blood urea level of 16-18 mg N/100 millilitres. Using a method similar to Weston and Hogan (1967), Vercoe (1968) demonstrated in cattle a maximum rumen ammonia level of 5-6 mg N/100 ml at a plasma urea concentration of 12 mg N/100 millilitres. A comparative study of urea recycling in cattle and sheep consuming equal amounts of a standard ration per unit of metabolic body size by Thornton (1970c) supported the findings of Weston and Hogan (1967) and Vercoe (1968). Thornton (1970c) suggested that the limit to the transfer of urea from the blood to the rumen fluid occurs at lower plasma urea concentrations and at slightly lower rumen fluid ammonia concentrations in cattle than in sheep.

It has been noted by various workers that dietary carbohydrate may exert some control over urea recycling from blood to the rumen. In experiments with cattle and sheep, Thornton (1970c) noted a decline in rumen fluid ammonia concentration associated with feed ingestion, while Packett and Groves (1965) demonstrated a decrease in the body urea pool of sheep during feeding. Both groups suggest that the energy supply associated with feed intake encourages a faster rate of incorporation of ammonia into microbial protein, resulting in the maintenance of a low rumen fluid ammonia concentration. The further

suggestion that this facilitates the transfer of urea from the blood to the rumen may lend support to the theory that rumen ammonia concentration is a controlling factor in urea recycling. The results of Houpt (1959) also showed an effect of carbohydrate on urea transfer, in that utilization of an injected urea load dropped from 52% to 22% when carbohydrate supplementation was withheld from the ration. That there was an effect of carbohydrate supplementation on nitrogen retention was recognized by Annison et al. (1954), even though the concept of urea recycling was not yet established at that time. Blackburn (1965) suggested that carbohydrate acts to slow the rate of ammonia absorption from the rumen by lowering the rumen pH, thereby providing rumen microbes more time and improved nutrient conditions for fixing ammonia into microbial protein. This theory would not necessarily rule out the possibility of increased endogenous recycling of urea due to lowered rumen ammonia concentration, but may well be a further elaboration.

Although of secondary importance to direct transfer of urea from the blood to the rumen, transfer of urea nitrogen via the salivary route may nevertheless be of a significant magnitude. McDonald (1952) suggested the importance of salivary nitrogen transfer to the rumen, while quantitative estimates of the amount of nitrogen transferred daily in saliva have been made by Hirose et al. (1960) in young steers and by Kay (1960) in sheep and young calves. Extensive research by Somers (1961a, b, c, d) yielded estimates of salivary urea concentrations, nitrogen concentrations, and flow rates in ruminants. While increased blood urea concentrations increased the salivary urea concentration, the non-linearity of the relationship suggested a

mechanism of transfer of blood urea to saliva other than passive diffusion. The exact nature of the transfer mechanism is still in question, particularly since results presented by Packett and Groves (1965) tended to support the findings of Bailey and Balch (1961 a, b) that urea does occur in ruminant saliva in proportion to the urea concentration in the blood.

An estimate of the relative importance of direct urea transfer across the rumen wall versus salivary urea nitrogen transfer has been made by Waldo (1968); he suggested that the direct transfer route is quantitatively more important by a factor of four to six times salivary transfer. A comparative study of urea recycling via both routes by Houpt (1959) indicated that direct transfer of plasma urea across the rumen wall might be of sixteen times greater magnitude, but Thornton (1970d) suggested that Houpt's calculations may seriously underestimate the contribution of salivary urea nitrogen to total urea recycling. Nevertheless, it would appear that direct transfer of blood urea across the wall of the digestive tract is quantitatively of greater importance than salivary transfer.

C. The Nitrogen Economy of Rangifer tarandus

It has been suggested that the species Rangifer tarandus is well adapted both morphologically and physiologically to survive the severe winter climate and difficult winter foraging conditions of the arctic tundra (Klein, 1970). In particular it would appear that caribou and reindeer are specially adapted physiologically to utilize both arboreal and terrestrial lichens. Studies by Nordfeldt et al. (1961) indicated that reindeer make more efficient use of lichen than either cattle or

sheep; reindeer appear to digest the crude fiber fraction especially well. Such studies also indicated however, that the nitrogen in lichens has a negative digestibility when lichens constitute the sole diet of reindeer (McEwan and Whitehead, 1970; Nordfeldt et al., 1961). Chemical analysis of the most preferred species of lichens utilized by reindeer and caribou indicated an average crude protein content of only 2-3%, with the nutritive value of the lichens remaining generally unimpaired throughout the winter (Scotter, 1965).

The significance of the low protein content of lichen becomes evident when the composition of winter feed of caribou and reindeer is considered. Although accurate information is limited, results of studies by Scotter (1967) suggested that at least 50% of the winter diet of the species consists of lichens, and some of the remainder of the diet may also be of relatively low protein content. The major value of lichens to reindeer and caribou probably results from the carbohydrate rather than the protein content. Lichens have a high starch content and are high in available energy (Klein, 1970; Scotter, 1965), which is quite important in terms of winter survival of caribou.

Adult reindeer and caribou exhibit a cyclical pattern of growth, characterized by weight gain in summer followed by weight loss in winter (McEwan and Whitehead, 1970). This is apparently a seasonal physiological change, likely related indirectly to reproductive processes (McEwan, 1968a), which adapts them to yearly variations in quality and quantity of food. In this way the period of highest metabolic demand occurs in spring and early summer when the nutritive quality of the naturally available forage, especially in terms of protein content, is highest (Klein, 1970). The winter period of near

physiological dormancy, in combination with declining quality of forage, causes the animals to voluntarily reduce feed intake and results in a cessation of growth and a reduction of metabolic rate (McEwan and Wood, 1961). The voluntary reduction in feed intake is particularly pronounced in bulls during the rut season. Lent (1965) reported that while barren-ground caribou bulls only reduce their feed intake during rut as their activity increases, reindeer bulls often do not feed at all during rut. Lack of food and continual activity often leave bulls in an emaciated and weakened condition at the end of the breeding season, just when winter stresses become severe.

In spite of the seasonal growth pattern and a greatly reduced winter metabolic demand for nitrogen, the loss of body weight in winter is not uncommon for free-ranging reindeer and caribou, particularly in young animals (McEwan, 1968a). McEwan suggests that one of the nutritional stresses affecting the winter weight loss in wild caribou calves could be the low protein to calorie ratio, particularly since lichen is very low in protein content and relatively high in energy.

It appears that virtually no investigations have been conducted on the nitrogen requirements of free-ranging caribou and reindeer, due to the technical difficulties involved. Nitrogen balance trials by McEwan and Whitehead (1970) on captive animals fed a complete, pelleted ration with a crude protein content of 21% have indicated that the amount of digestible nitrogen required for nitrogen equilibrium amounts to 0.462 g $N/kg^{0.75}$ per day, a value fairly comparable to that reported for cattle and sheep (Brody, 1945; Harris and Mitchell, 1941; Moir and Williams, 1950). These trials on caribou and reindeer indicated that nitrogen retention decreased by 15-20% in winter

compared to summer, as voluntary feed intake decreased. However, as the authors suggest in their paper, extrapolation of these nitrogen requirement figures to free-ranging animals is questionable. It has been proposed (Elliott and Topps, 1962) that nitrogen requirements for maintenance are dependant on the composition of the diet, so that increased proportions of ground roughages in the diet result in a substantial increase in digestible nitrogen required for nitrogen equilibrium. What effect the high fiber and starch content of lichen (Scotter, 1965), combined with the low protein content of the forage, would have on the dietary nitrogen requirement for free-ranging animals is a matter of conjecture.

There appears to be little consideration in the literature of the possible contribution that recycled blood urea nitrogen might make toward fulfilling the winter nitrogen requirements of reindeer and caribou, under either captive or free-ranging conditions. Studies of barren-ground caribou (McEwan, 1968b) indicated that captive caribou consuming a ration with a crude protein content of 21% had an average plasma urea nitrogen concentration about 28% higher than that of wild caribou. However, the average plasma urea concentration of approximately 22 mg/100 ml reported for wild caribou was determined from blood samples taken in July, when the protein content of tundra forage is highest. Extrapolation of this value to winter conditions may, or may not, be justified. Therefore the plasma urea concentrations in wild caribou during winter may be substantially lower than the figures presented by McEwan (1968b).

EXPERIMENTS AT THE UNIVERSITY OF ALBERTA AND THE UNIVERSITY OF BRITISH COLUMBIA

A. General Experimental - Materials and Methods

1. Outline

The experiments herein reported were conducted from June of 1970 through June of 1971. All trials conducted on the species Rangifer tarandus[1] were carried out under the auspices of Dr. E.H. McEwan of the Canadian Wildlife Service, at the Zoology Vivarium, University of British Columbia. The first trials were conducted in the early summer of 1970, while the experimental animals were in the summer growth phase of their growth cycle. Later trials were conducted in February and March of 1971, when the animals were in a winter dormancy phase.

Experiments conducted on beef bulls and reported in this thesis, were carried out during May and June (1971) at the Edmonton Research Station, University of Alberta.

2. Technique Used

The technique used to estimate the extent of urea recycling in both caribou and beef bulls was one developed at the University of Alberta by A.L. Ford (Ford, 1969; Ford and Milligan, 1970).

Basically, the technique involves the labelling of the body urea pool with a single injection of ^{14}C-urea, with subsequent total collection of the urine for 48 hours. For animals maintained in a steady, fed state, the urea influx to the body pool equals efflux, where the two major possible exits from the urea pool would be urea

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[1] Rangifer tarandus refers to both caribou and reindeer. Subspecific names are sometimes seen, but are not used in this thesis because the origin of the introduction of reindeer into Canada is rather vague.

excretion in the urine and urea transfer from the blood plasma into the digestive tract where the urea is hydrolized to CO_2 and ammonia. Therefore, when the urea pool is labelled with a tracer dose of ^{14}C-urea and all the label that is excreted in the urine is collected, the difference between the amount of label injected and the amount of label collected in the urine is considered to equal the amount of label that left the plasma and entered the digestive tract. If the total amount of urea excreted over the same period of time is also measured, then the amount of urea irreversibly entering the digestive tract can be estimated by difference, after relating the amount of urinary urea collected to the percentage of injected label recovered in the urine. The equation used is:

$$R = \frac{1 - L}{L} \times U$$

where: R = the grams urea recycled per day

U = grams urea excreted in the urine per day

L = the fraction of injected label recovered in the urine

For further development of the technique see Ford (1969).

3. Injection of Label

The tracer solution of ^{14}C-urea[2] was made up in 0.15 M NaCl to give an activity of approximately 22×10^6 dpm/millilitre. A volume of 10.00 ml was injected into the jugular vein, via syringe and needle in the case of the caribou, and via a jugular catheter in the case of the beef bulls. Where a jugular catheter was used, it was flushed immediately after injection with 10-20 ml of 0.15 M NaCl.

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[2] The Radiochemical Center, Amersham, England.

4. Collection of Blood

Venous blood samples were collected twice daily during trials, at somewhat random times. Approximately 8 ml samples were collected using 10 ml, heparinized Vacutainers[3]. The first blood sample was taken immediately prior to the injection of label; subsequent samples were taken alternately from either right or left jugular vein, to minimize trauma.

In the case of the beef bulls, an attempt was made to obtain blood samples from a permanent jugular catheter, but this method had to be discarded.

5. Treatment of Samples

a) Blood

Blood samples were centrifuged in the Vacutainer tubes at 1240 x gravity for a minimum of 15 minutes. The plasma was withdrawn, divided into duplicate vials, and frozen.

b) Urine

In all trials the urine volume was measured immediately after each collection period, and duplicate samples of 20 ml each withdrawn for later analysis. These samples were refrigerated immediately and then frozen at the earliest opportunity.

c) Feed

Random feed samples were taken periodically and analysed for crude protein content by the Kjeldahl method (AOAC, 1965). Feed

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[3] Beckton, Dickinson and Co., Canada Ltd., Clarkson, Ontario, Canada.

samples were stored cold in polyethylene bags until analysed.

6. Transport of Samples

All blood and urine samples collected at the University of British Columbia were sent back to the University of Alberta for determination of urea content. Therefore, as soon as possible after collection, these samples were frozen and stored until the date of shipment. In the case of both the summer and the winter series of trials, all samples were maintained in duplicate to allow, as a safety precaution, for shipment of a complete set of samples on each of four separate air flights. Each shipment was securely packed in polystyrene shipping containers and, through the use of dry ice, maintained in a frozen condition for the duration of transport. All samples were immediately unpacked upon receipt at the University of Alberta, and kept frozen until analysed for urea. One set of samples was temporarily delayed in transit and was therefore received at the University of Alberta in a partially thawed condition. Comparison of results of urea determination on this set of samples with the appropriate duplicate set indicated no difference in urea concentrations that were detectable by the method of analysis used.

7. Urea Determination

The urea concentration of the plasma and urine was determined using the method of Fawcett and Scott (1960) with the following modifications. Sodium nitroprusside, sodium hypochlorite and buffered urease solutions were prepared just prior to each urea determination sequence. The urease preparation used was Worthington precrystalline

urease[4] (URPC, 240 units/mg). Urease incubation was carried out at room temperature for 30 minutes. Standards were included in each determination sequence. All samples were analysed in duplicate. Plasma was diluted 1:100 while urine was diluted 1:2000. All water used in the preparation of reagents, in dilutions and in washing glassware was demineralized by passing distilled water through a Bantam[5] Demineralizer column (standard cartridge). A random check for ammonia content of urine samples was made in each urea determination sequence, and in all cases the free ammonia concentration of urine proved negligible.

8. ^{14}C Measurement

Urine from each collection period (0.20 ml) was added to 15 ml of the scintillation medium of Jeffay and Alvarez (1961) in a scintillation vial. All samples were prepared in duplicate. After mixing, the samples were counted in a Nuclear Chicago Mark I Liquid Scintillation System at 10°C for four minutes; each vial was counted three times. Samples collected at the University of British Columbia were analysed at the end of each trial, utilizing the facilities of the Department of Zoology. The ^{14}C content of the injection solution was determined in triplicate within two hours of the start of each trial. A volume of 0.01 ml of tracer solution was counted in 15 ml of scintillation medium. Counting efficiency was determined by the channels ratio method (Bruno and Christian, 1961; Hendler, 1964). It was found that the efficiency of counting on the University of British Columbia

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[4] Worthington Biochemical Corporation, Freehold, New Jersey.

[5] Barnstead Still and Sterilizer Co., Boston, Massachusetts.

machine was usually about 58%, which was typically 5-9% lower than the efficiency of counting on the machine used at the Department of Animal Science, University of Alberta. Investigations revealed that, following ^{14}C-urea administration, the portion of ^{14}C present as bicarbonate or carbonate in the urine of caribou and beef bulls was negligible.

B. Experiments on *Rangifer tarandus*

1. General Outline

a) Facilities

Experiments on the extent of urea recycling in caribou were conducted at the Zoology Vivarium, University of British Columbia. The facility there consists of a laboratory building and an animal housing unit. The animal unit is a roofed, shed type structure left partially open to the outside environment, and is fully described by Wood et al. (1961). The caribou are presently penned individually in stalls with slatted wood floors measuring 20 ft x 4 feet.

b) Animals

Two mature bull animals of the species *Rangifer tarandus* were used in these trials. In actual fact one animal (R4) was a reindeer while the other animal (X4) was a caribou. Both of these animals had been handled and subjected to metabolic trials previously, and were reasonably tractable.

c) Feed

The major portion of the diet fed both animals consisted of a pelleted ration no. 36-69c (Table 1). In some trials dried lichen was also included in the diet. Approximately 400 kg of freshly picked

Table 1 Formulation for caribou bull ration no. 36 - 69c

Constituent	Pounds/ton
Ground yellow corn	630
Ground no. 5 feed wheat	525
Ground wheat bran	275
Molasses (cane)	100
Ground beet pulp	200
Dehydrated grass meal	200
Dairy (calf) micromix *	50
Chromic oxide	20
	2000

*Micromix contains

Calcium	20 %
Phosphorus	4 %
Salt (NaCl)	40 %
Vitamin A	160,000 I.U./pound micromix
Vitamin D2	120,000 I.U./pound micromix
Vitamin E	240 I.U./pound micromix
Chelated trace minerals	

lichen were obtained in the fall of 1970 from Canadian Wildlife Service personnel stationed at Inuvik, Northwest Territories. Upon receipt at the University of British Columbia, the lichen was sun dried and stored, in preparation for the winter trials to be conducted in early 1971. The protein and energy content of both lichen and ration no. 36-69c are shown in Table 2. Both animals had previously been maintained solely on ration no. 36-69c for more than one year of time, and were therefore well adapted to this diet.

d) Metabolism crate

The metabolism crate used for conducting all caribou trials consisted of a raised stall of dimensions 4 ft wide x 10 ft long x 4 ft high, with a feed bin and a water bowl at opposite ends. The feed bin was located at the gate end of the cage and was removable. The water bowl was positioned at the other end and outside the actual cage to avoid water contamination of urine. A raised V-board at each end and studs on the sides prevented the animal from jumping out.

The floor consisted of a heavy wire mesh screen supported by periodic floor joists, which would support the weight of the animal yet allow urine to fall through. A small gauge, removable wire screen below this floor collected the fecal pellets, while allowing the urine to fall through to a slanted, V-shaped collecting trough. The wire screen was removed and cleaned daily. A removable ramp, complete with traction slats, was used to run the animal up into the metabolism crate. The major features of the metabolism crate are illustrated in Figure I.

e) Water

Animals were at all times allowed free access to water from a hog

Table 2 Energy and nitrogen values per unit dry matter for ration no. 36 - 69c and lichen

Parameter measured	Value for	
	no. 36-69c	lichen
Moisture, %	9	10
Gross energy*, kcal/g	4.5	4.2
Apparent digestible energy kcal/g	3.3	2.8
Nitrogen, mg/g	19.2	3.2
Crude protein, % (approximate)	12.0	2.0
Chromic oxide, %	0.5	

*Energy values supplied by Dr. E.H. McEwan, Canadian Wildlife Service

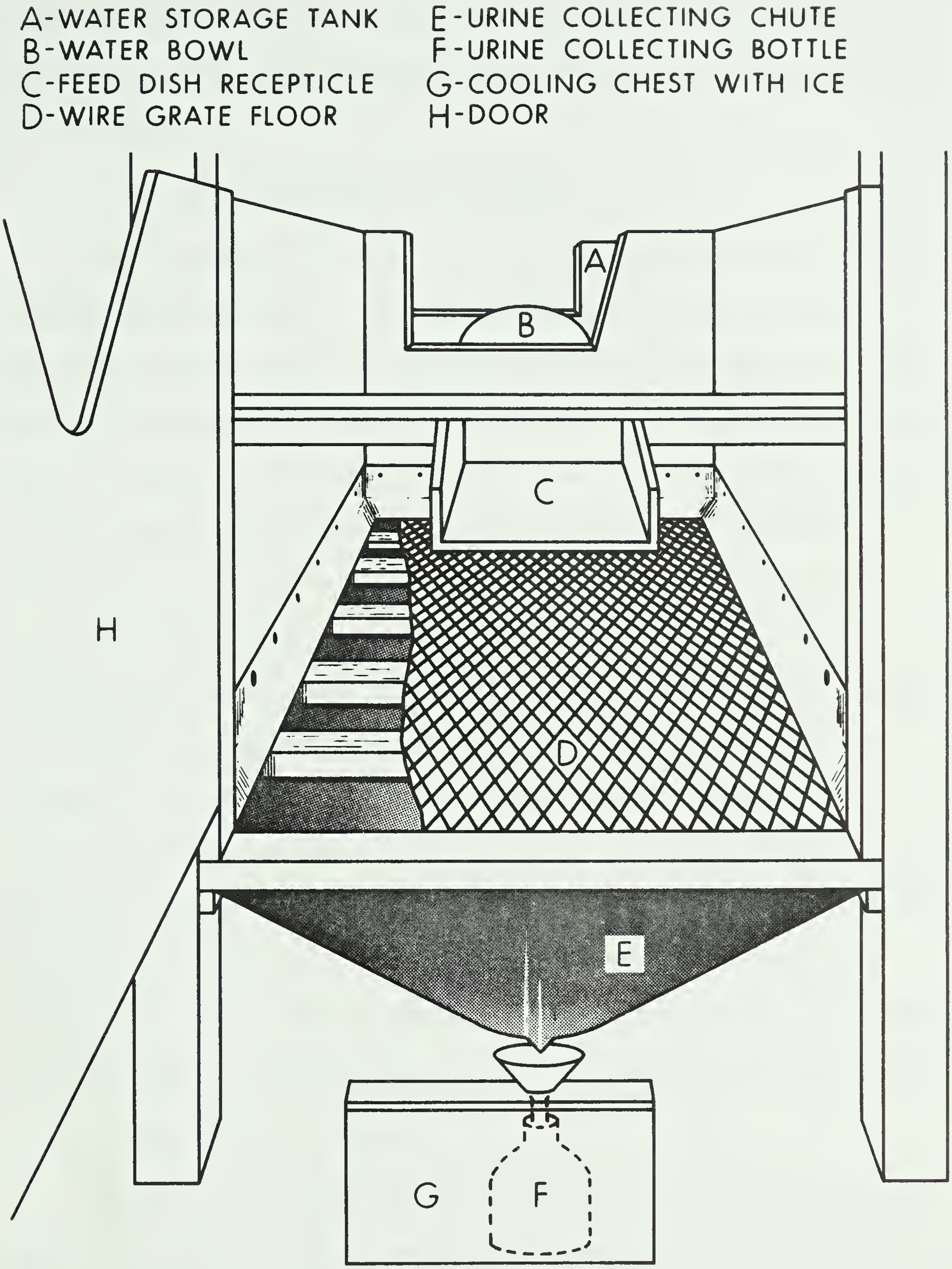

Figure I. Metabolism crate and urine collection system for caribou.

bowl equipped with an automatic float shut-off. Water was supplied to the bowl from a 20 l carbuoy, such that daily water intake could be measured.

f) Urine collection and treatment

Urine issuing from the collection trough was collected in a 3.4 l plastic milk container. The milk container was situated inside a polystyrene type camp cooler and surrounded with crushed ice in order to cool the urine to a temperature approaching 0° Centigrade. A large plastic funnel was used to convey the urine into the storage container. A thick wad of cheezecloth in the bowl of the funnel effectively filtered out any hair or debris not caught in the screens. Urine collection containers were changed at intervals of 6 to 9 hours, except during Trial I when they were changed more frequently in order to establish the label recovery pattern. After urine volume was measured, the aliquot withdrawn for label and urea analysis was filtered through absorbent cotton to give a very clear sample. Initial tests were conducted to establish if label was lost from the collection container as $^{14}CO_2$, as a result of urea cleavage catalysed by any contamination with urease. Cooling the collected urine with crushed ice was found to effectively and completely inhibit any loss of label.

2. Summer Growth Trials

The first set of four trials on the caribou bulls was run from July 6 to July 23 of 1970. These trials were designed to estimate urea recycling in mature bulls during the summer growth period. An attempt was made, through limitation of the amount of ration provided, to approximate the intake of gross energy and protein that feral

animals might be expected to ingest at that time of year.

a) Environmental conditions

All trials were conducted in the animal shed, but the construction of this unit allowed for access of outside air. Outdoor temperatures were generally fairly warm for the coastal climate (18-27°C) and although completely shaded, the test animals did suffer somewhat from heat and flies. Night temperatures were noticeably cooler and did afford relief to the animals. The facilities available did not allow for any control of the test environment.

b) Weight and condition of animals

Both experimental bulls were established on a fairly high intake of ration no. 36-69c before the trials were initiated. The reindeer bull (R4), when first measured, was gaining weight rapidly, and at the beginning of Trial I weighed 204 kilograms. The caribou bull (X4) was slower to attain a high intake level and at the start of Trial IV (July 21) weighed only 151 kilograms. It was decided to reduce the intake of R4 somewhat in order to decrease his rate of gain and thus hopefully more nearly approach natural levels of protein intake.

R4 in particular was beginning to develop the heavy neck muscling associated with the onset of rut. Both animals had a full-sized set of antlers which were, however, still in the velvet stage.

c) Feeding regime

Both animals were given a daily total of 4000 g of pelleted ration no. 36-69c and were offered this amount at least one week before each trial. Total daily feed was divided into 8 portions of 500 grams.

One portion was provided every three hours. This procedure was followed to establish a steady, fed state, with a view to stabilizing the body urea pool.

3. Winter Dormancy Trials

The second set of five trials on the caribou bulls was conducted from February 23 to March 18 of 1971. These trials were designed to estimate the extent of urea recycling in mature bulls during the winter maintenance period of the growth cycle which follows the rut season. It was not possible to completely approximate the theoretical intake of only 2% crude protein which feral animals may face, but feed intake and protein levels were drastically reduced from summer levels.

a) Environmental conditions

Still air temperature inside the animal housing unit ranged from -4 - +10° Centigrade. While freezing temperatures at times played havoc with watering facilities, the test animals themselves evidenced no noticeable ill effects, and in fact appeared more comfortable than they had during the summer heat.

b) Weight and condition

Both experimental animals were just beginning to regain their appetites following the period of low intake during the rut season. Both animals were in a very emaciated condition, without any evidence of the heavy muscling and fat of the pre-rut period. R4 had shed his antlers on November 17 of 1970 and was now well started on a new set. Relative to the caribou bull (X4) and probably animals in the wild, R4 was at least one month in advance of the normal cycle (E.H. McEwan,

personal communication). Both animals weighed approximately 130 kg throughout this experimental period, so that relative to previous summer weights, R4 had lost more body weight.

c) Feeding regime

About one month prior to the beginning of the trials, the amount of pelleted ration no. 36-69c allotted each animal was gradually reduced and dry lichen substituted in its place. Bull R4 consumed the lichen quite readily if nothing else was available; bull X4 was less willing to consume lichens. At the commencement of trials, and throughout the trials, R4 received a total of 1615 g of ration no. 36-69c per day, augmented with an amount of lichen governed by his own voluntary intake. This averaged 1400 g of lichen per day. The pelleted ration was fed in two equal portions at 900 h and 1800 h; lichen was offered in 500 g portions at 3-4 h intervals throughout the remainder of the 24 h period. The weights of all feed components consumed were measured. The same regime was followed with bull X4 except that the amount of ration 36-69c was increased to 1865 g prior to Trial X in order to maintain body weight. The average daily intake of lichen by X4 was only 800 grams. Average daily feed, nitrogen and water intakes for each trial are presented in Table 3.

C. Experiments on Beef Bulls

Experiments on the extent of urea recycling in yearling beef bulls were conducted at the Edmonton Research Station, University of Alberta. The animals were housed indoors under continual lighting.

1. Animals

Two yearling beef bulls from the University of Alberta Ranch at Kinsella were used. Both animals were 62.5% Angus x 37.5% Galloway crossbreds, by the same sire, and were designated #324 and #328. The animals were handled and allowed to adjust to the environment for about one month before the trials were initiated.

2. Feed

Both animals were obtained directly from a feedlot trial and were in fat condition. The feed used for our trials was a mixed oat hay of poor quality containing approximately 8% crude protein. This chopped ration was fed ad libitum for at least one month prior to the initiation of trials, to ensure complete adaptation to the ration. A mixture of 80% cobalt-iodized salt and 20% dicalcium phosphate was available to the bulls at all times. Bull #324 lost 28 kg and bull #328 lost 29 kg during this period, so that at the start of the reported trials they weighed 302 kg and 301 kg respectively. During the trials a daily total of 6 kg of the oat hay was offered to each bull in 6 equal portions at 4 h intervals, in order to establish reasonably continuous intake. It was estimated that an intake of approximately 6 kg of feed per animal per day, calculated on a total protein basis, would meet maintenance requirements (National Research Council, 1970). The actual measured, voluntary feed intake averaged 5.5 kg per animal per day, which may have been slightly below minimum maintenance requirements. Both animals continued to lose 0.5 kg per day throughout the course of the trials.

3. Pen and Feeding Arrangements

Animals were individually penned in crates which were large enough to allow the bulls to lie down. Each animal was held secure with a dairy stanchion bolted to the front of the crate. A large bin for feed was easily accessible to each animal. Water was available at all times from a large bucket, which allowed for estimation of daily water intake. The two crates were placed side by side.

4. Urine Collection

A male urine collection device as described by Ford (1969) was used. Figure II from Ford (1969) illustrates this piece of equipment. The rubber tubing carried the urine by gravity flow to the collection and cooling apparatus previously described. The funnel and filter equipment were not used in this case, nor were the final urine samples filtered through absorbent cotton. In all other respects urine collection and sampling were similar to the caribou trials.

5. Label Injection and Blood Collection

It was decided that a jugular catheter might conveniently be used for both ^{14}C-urea injection and blood sample collections, since these animals were quite docile. External catheterization on one side of each animal was performed, just prior to the first trials, using a 13 gauge hypodermic needle and Intramedic[6] Polyethylene tubing (PE - 90/S36"). Label was injected and the tubing flushed with heparinized saline by means of a 3-way stopcock[7]. In each case the

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[6] Clay-Adams Inc., New York, U.S.A.

[7] The Stevens Companies, Physicians and Hospital Supplies, Toronto, Winnipeg, Calgary, Vancouver.

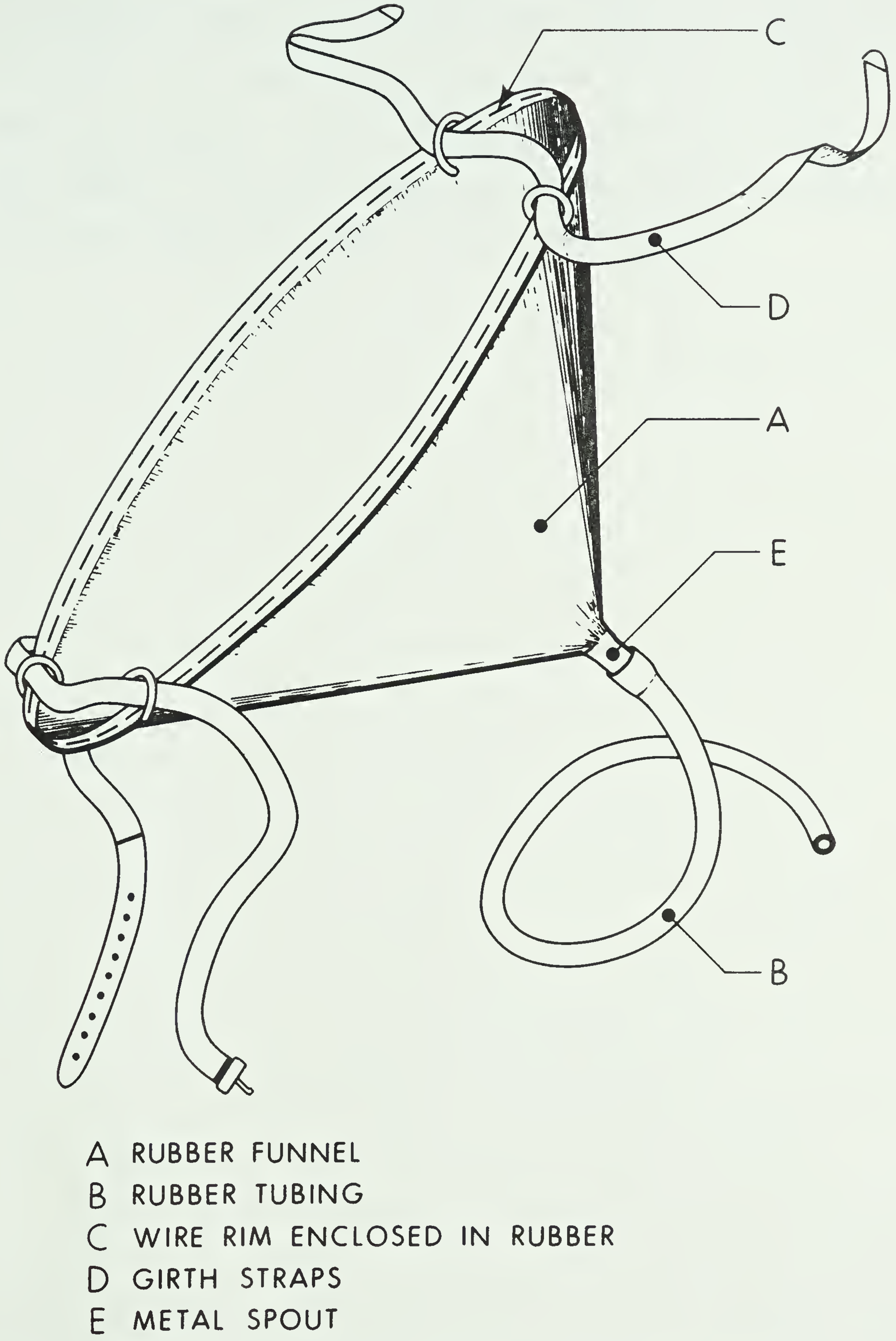

Figure II. Male urine collection device used on beef bulls (from Ford, 1969, p. 27).

first blood sample was taken with a heparinized 10 ml syringe prior to injection of label. The catheters were left in for future blood collections, but in both cases had to be removed due to abrasive damage and plugging. The subsequent use of Vacutainers proved satisfactory, so that in a second trial on bull #328, the catheter was removed immediately after injection of ^{14}C-urea.

RESULTS

A summarization of animals and trial treatments is presented in Table 3. An attempt has been made in the last column to relate the approximate nitrogen intake to the metabolic body size of the animal during each trial. In each case, the figure presented is on the basis of the total nitrogen intake, because ration digestibilities, where available, were determined on different animals fed a different preparation of the ration (see Table 2). The data presented in Table 3 indicate that, on a metabolic body size basis, nitrogen intake of the caribou was reduced by at least one third in winter versus summer trials. The data also suggest that the nitrogen intake of the beef bulls was as low as, or lower than, that of the caribou in winter.

Figure III shows the variation in blood plasma urea concentrations over the course of some selected trials. The curve of a typical trial for each of the experimental treatments is presented. In all cases plasma urea concentrations remained relatively constant and well within the range of variation reported by Lewis (1957) for a steady, state circumstance in sheep. The plasma urea concentrations of the beef bulls were quite low compared to the caribou, with an average of 8.5 mg/100 millilitres. The plasma urea concentrations of the caribou bulls tended to be slightly higher in winter than in summer, even though nitrogen intake was significantly lowered in winter.

Figure IV shows the pattern of the excretion of urea over the duration of a typical trial. Urine flow rate and urinary urea concentration appeared to vary inversely to produce a relatively constant rate of excretion of urea (Table 4). Within any particular trial, urea excretion was dependent upon, but was not a linear function of,

Table 3. Animals and trial treatments for urea recycling experiments

Trial no.	Animal	Ration	Intake (g/day)	Protein content (%)	Nitrogen intake (g/day)	Water intake (1/day)	Animal weight (kg)	N intake per Kg 3/4 (g/day)
1.	R4	36-69c	4000	12	77	11.7	204	1.4
11.	R4	36-69c	4000	12	77	13.7	205	1.4
111.	R4	36-69c	4000	12	77	15.6	207	1.4
1V.	X4	36-69c	4000	12	77	15.6	151	1.7
V.	R4	36-69c lichen	1615 1465	12 2	36	12.2	130	0.94
V1.	R4	36-69c lichen	1615 1490	12 2	36	8.3	130	0.94
V11.	X4	36-69c lichen	1615 920	12 2	34	11.3	130	0.88
1X.	R4	36-69c lichen	1615 1590	12 2	36	10.1	130	0.94
X.	X4	36-69c lichen	1865 720	12 2	38	7.7	130	0.99
X11.	324	oat hay	5500	8	70	22.0	300	0.97
X1V.	328	oat hay	5500	8	70	18.2	300	0.97

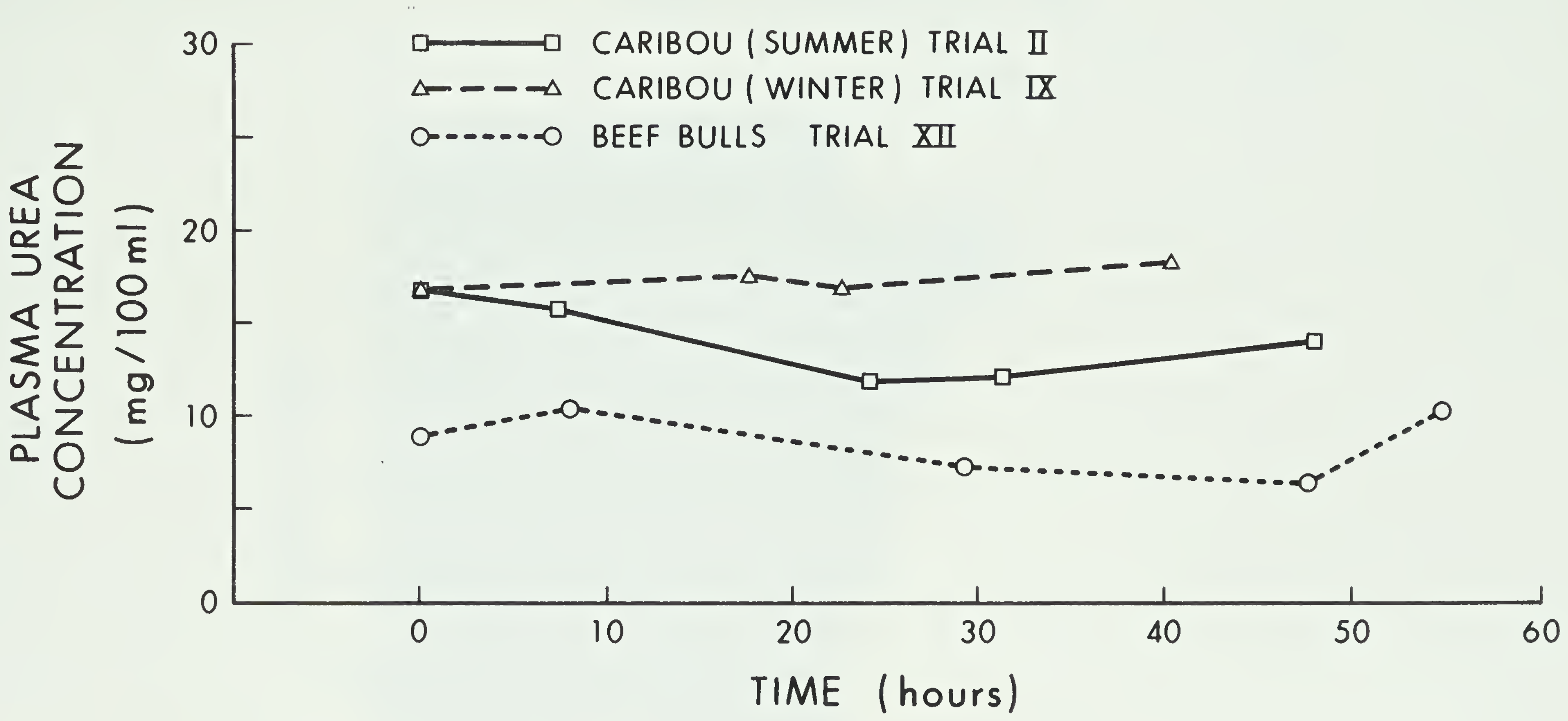

Figure III. Plasma urea concentration over time - typical trials.

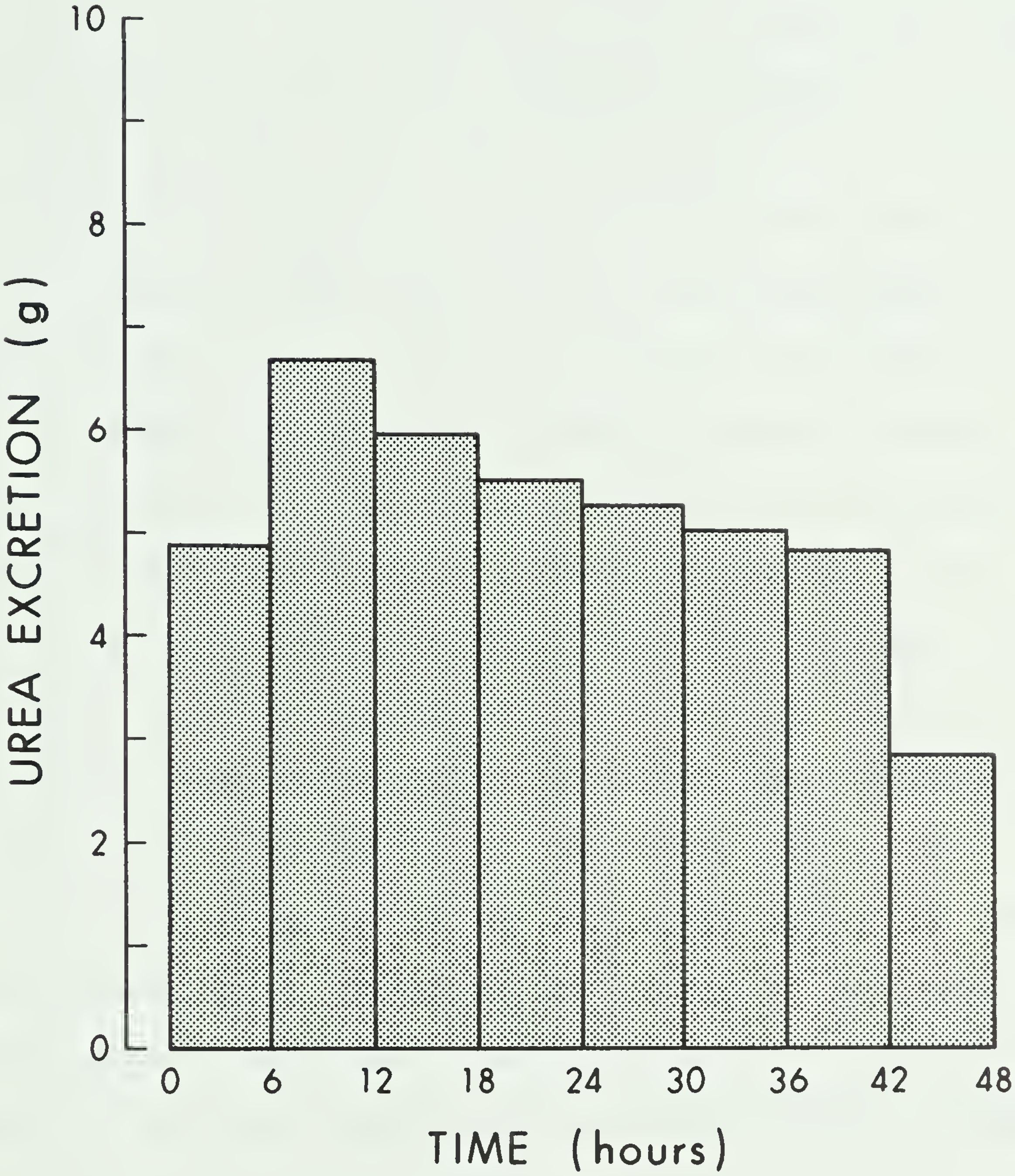

Figure IV. Urea excretion rate of typical trial (II).

urine flow rate. A fall in flow rate was generally accompanied by an increased urine urea concentration, but not proportionately so.

A typical pattern of ^{14}C recovery in the urine is presented in Figure V, where the specific activity of urinary urea for Trial I is plotted against time. This particular trial is demonstrated because the urine collection vessels were changed more frequently in this trial than in the others, thus, the curve obtained is more definitive. Figure V indicates a completeness of ^{14}C recovery at 48 hours. In actual fact however, before urine collection was discontinued the ^{14}C activity of urine collected at 48 h was measured by the method previously described. Collection was considered adequate to ensure complete recovery of label only when urine urea activity approached background levels. In both trials conducted on animal X4, that is Trial VII and Trial X, it was necessary to collect for longer time periods (69 h and 62 h, respectively). In trials XII and XIV, using the beef bulls, urine was collected for 56 and 60 hours respectively, as complete label excretion for some reason took longer in these animals also. In any event, the recovery curve presented in Figure V is representative of all trials, except in the extent of tailing at the lowest specific activities. Trial XI, conducted on beef bull #328, is not reported because complete urine collection was not achieved.

The experimental results relating to urea kinetics are summarized in Table 4. The urea recycling data for both caribou and beef bulls under all trial treatments are presented. The column headed urea flux represents the total amount of urea passing through the body pool daily, that is, it is the sum of urea excreted plus urea recycled. There was a constancy of daily urea excretion between trials in any

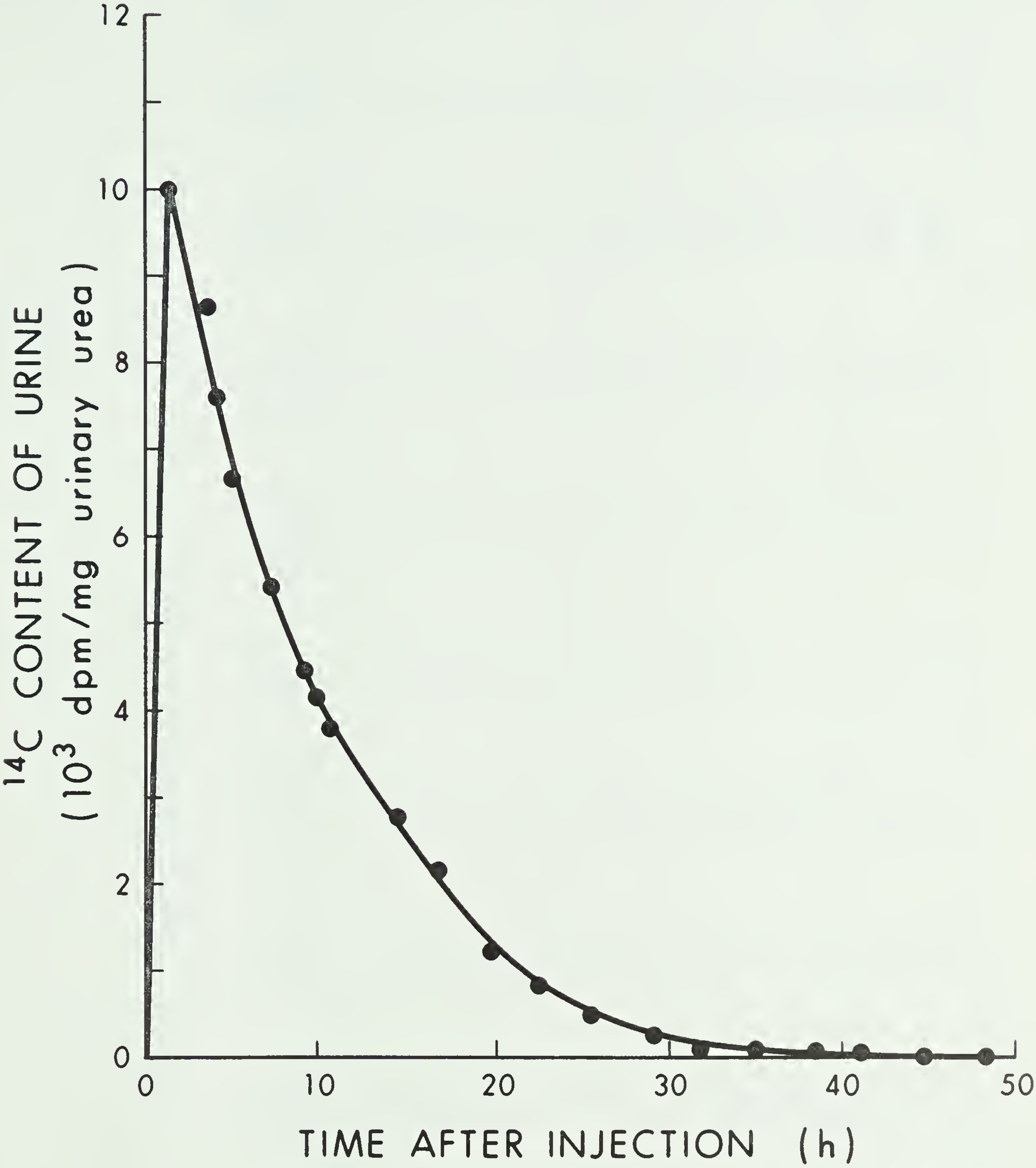

Figure V. Label recovery pattern in urine - plot of urinary urea specific activity versus time.

Table 4. Summary of urea recycling data for all trials

	Trial no.	Plasma urea (mg/100ml)	Urine urea (mg/100ml)	% of ^{14}C recovered in urine	% of ^{14}C recycled	Urea excretion (g/day)	Urea recycled (g/day)	Urine flow (ml/day)	Urea flux (g/day)	Urea recyl. per kg 3/4 (g/day)
Caribou summer trials	I.	15.8	464	52.4	46.6	23.5	21.4	5076	44.9	0.40
	II.	14.2	324	51.1	48.9	20.5	19.6	6300	40.1	0.36
	III.	15.9	296	38.3	61.7	22.2	35.7	7502	57.9	0.66
	IV.	17.1	594	55.1	44.9	22.6	18.4	3804	41.0	0.34
Caribou winter trials	V.	18.0	293	36.9	63.1	17.9	30.6	6105	48.5	0.79
	VI.	18.1	351	42.2	57.8	18.5	25.3	5265	43.8	0.65
	VII.	20.9	879	43.0	57.0	17.7	23.5	2012	41.2	0.61
	IX.	17.4	230	40.3	59.7	17.4	25.7	7560	43.1	0.66
	X.	19.2	472	47.9	52.1	18.5	20.1	3919	38.6	0.52
Beef bulls	XII.	8.6	653	56.2	43.8	25.0	19.5	3826	44.5	0.27
	XIV.	8.5	563	48.2	51.8	25.1	26.9	4450	52.0	0.37

one treatment series. The average of urea excretion in Trials I through IV was 22.2 $\pm$ 1.3 g per day, with a standard error of the mean of 0.63. During the winter trials with caribou (V - X), the average of urea excretion was 18.0 $\pm$ 0.5 g per day (S.E. = 0.22). Daily urea excretion by the two beef bulls was virtually identical to one another. This data, coupled with the relative uniformity of plasma urea concentrations for trials within any one treatment, suggests that standardization of treatments was attained. Since urea excretion remained constant from trial to trial in any one treatment series, variation in urea flux is, for the most part, a reflection of changes in the amount of urea recycled daily. The quantity of urea recycled daily was calculated using the equation presented on page 14.

Variability between trials within a treatment series in the proportion of plasma urea recycled was somewhat greater than expected, but in each case it was due in large measure to one divergent trial. This appears to be particularly true in the case of Trial III, where the percent of ^{14}C-urea recycled was very high relative to the other caribou summer trials.

Of particular interest are the comparisons of treatment group averages between caribou summer trials and caribou winter trials, between caribou summer trials and beef bull trials, and between caribou winter trials and beef bull trials. Such comparisons are presented in Table 5, where the percent of label recycled is used as the term of reference. The data has been statistically treated utilizing the t-test for comparison of two sample means with unpaired observations and equal variances (Steel and Torrie, 1960). The standard 5% and 1% significance levels were used, although the small number of trials

Table 5 Percent urea recycled--t-Test between treatment averages

Caribou (summer) Trial no.	Caribou (summer) % recycled	Caribou (winter) Trial no.	Caribou (winter) % recycled	Beef bulls Trial no.	Beef bulls % recycled
1.	46.6	V.	63.1	X11.	43.8
11.	48.9	V1.	57.8	XiV.	51.8
111.	61.7	V11.	57.0		
1V.	44.9	1X.	59.7		
		X.	52.1		
Average	48.6	Average	57.9	Average	47.8
Average Trial 111 omitted	46.8				

Comparison between	Difference between averages	Calculated "t"	Significance
Caribou (winter) & caribou(summer)	9.3	2.56	*
Above-Trial 111 omitted	11.1	4.37	**
Caribou(summer) & beef bulls	0.8	0.16	--
Caribou(winter) & beef bulls	10.1	2.74	*
Caribou(summer) & sheep	7.4	1.59	--
Caribou(winter) & sheep	16.6	4.39	**
Sheep & beef bulls	6.5	1.10	--

* differences significant ($P<0.05$)
** differences significant ($P<0.01$)

somewhat limits the number of degrees of freedom, such that a higher level of significance might appropriately have been chosen (Steel and Torrie, 1960).

The data in Table 5 suggest that there is a positive significant difference in the proportion of blood urea recycled by winter caribou as opposed to summer caribou, at the 5% significance level. When the results from Trial III are omitted from the calculations, a positive difference is evident at the 1% significance level. In the calculation of these results, it was reasoned that since only one summer trial was conducted on animal X4, the value obtained for percent urea recycled in that one trial should be weighted equally with the average of all summer trials on animal R4. Similarly, a significant difference at the 1% level occurs when the proportion of blood urea recycled is compared in winter caribou versus sheep.

The sheep data used here is taken from Ford (1969), and is presented more fully in Table 6 and Table 7. Only those trials considered applicable for comparison with the present work have been used. Table 5 further indicates a positive significant difference between winter caribou and beef bulls at the 5% significance level. In all other instances of comparison, no significant differences in the percent of label recycled are apparent.

Looking at the column headed urea recycled per $kg^{3/4}$ (Table 4), it is noted that on a metabolic body size basis, the winter caribou appeared to recycle an appreciably greater quantity of urea than did either summer caribou, or beef bulls. The winter caribou also recycled a greater daily quantity of urea per unit of metabolic body size than did the sheep of Ford's (1969) study. The sheep data

presented in Table 6 indicate that the total nitrogen intake of the sheep per unit of metabolic body size was reasonably similar to that of the winter caribou (Table 3).

Table 6 Trial treatments for urea recycling experiments on sheep[1] (adapted from Ford,1969)

Trial no.	Sheep	Ration	Quantity eaten (g/day)	Water infused (ml/day)	N. intake per kg3/4 (g/day)
10,12,13	ewe	hay	725	1570	0.78
16	ram	hay	920	1570	0.85
32	ram	hay	1000	3100	0.92
33,34	ram	barley	500	1570	0.54+

[1] The weight of the ram was constant at 42.2 kg., while that of the ewe was constant at 34.0 kilograms.

Table 7. Summary of urea recycling data for sheep (adapted from Ford, 1969)

Trial no.	Plasma urea (mg/100ml)	Urine urea (mg/100ml)	Urine flow (ml/day)	Urea excretion (g/day)	% of ^{14}C recovered	% of ^{14}C recycled	Urea recycled (g/day)	Urea flux (g/day)	Urea recycl. per Kg 3/4 (g/day)
10	21.6	1495	620	9.27	56.7	43.3	7.07	16.34	0.50
12	18.6	1017	1101	11.2	49.9	51.1	11.25	22.45	0.80
13	18.3	945	966	9.04	58.6	41.4	6.41	15.44	0.45
16	33.6	1520	932	14.2	52.4	47.6	12.9	27.1	0.78
32	15.8	426	2619	11.16	57.2	42.8	8.33	19.49	0.50
33	21.7	570	1882	10.73	65.3	34.7	5.70	16.43	0.34
34	21.8	1400	1116	15.62	71.6	28.4	6.19	21.81	0.38
						Average 41.3			Average 0.53

DISCUSSION AND CONCLUSIONS

When viewing the data presented in this thesis, it is important to be aware of one rather subtle consideration; factors which influence or determine the blood plasma urea level were not specifically studied. The ^{14}C tracer technique developed by Ford (1969) only allows an estimate of the proportion and amount of plasma urea recycled to the digestive tract at any particular plasma urea concentration.

A further distinction can be made between the studies of Ford (1969) and the work reported here. Ford varied the blood urea levels in different trials in order to establish the relationship between plasma urea concentration and urea recycled to the digestive tract. However, in any one trial the blood urea concentration was maintained in a steady state and no real attempt was made to study the factors involved in attaining that particular blood urea concentration. In the trials conducted using caribou and beef bulls, the plasma urea concentrations were not manipulated, so that plasma urea levels were relatively low under all treatments. The purpose of the trials was to simulate the forage conditions that free-ranging animals might be subjected to at different times of the year, and to estimate the extent to which urea recycling occurs at the blood urea concentrations attained under such nutritional situations.

The data presented in Table 4 and Table 5 suggest that, during the summer trials,the caribou recycled a proportion of the plasma urea similar to the proportion recycled by the beef bulls. There was a tendency for the percent plasma urea recycled to be higher for summer caribou and beef bulls than for sheep, but this tendency was not statistically significant (Table 5). It is worthwhile to keep in

mind certain further considerations. Firstly, the metabolic demand for nitrogen for purposes of tissue regeneration, antler development and rut muscle development is probably quite considerable in caribou at this time of year, even in mature bulls. Similarly, the high growth potential of young beef bulls implies the capacity for extensive protein deposition and therefore the metabolic maximization of nitrogen utilization. In both cases it is not unreasonable to suggest that, since relatively low protein diets were offered in these trials, urea recycling might have occurred to near maximum levels. On the other hand, the sheep used in Ford's (1969) experiments may not have been recycling urea to their maximum potential, since they were mature animals with minimal requirements for protein synthesis.

Furthermore, only one successful trial was run on each of the two beef bulls, with rather divergent results. Thus the average value for percent of the plasma urea recycled by the beef bulls can be construed only as indicative of the possible range of urea recycling in cattle. The range indicated by these trials does not approach the range of 59-90% reported by Mugerwa and Conrad (1971). The technique employed by these researchers was based on the principles of first-order kinetics. Analysis of the results was, therefore, not strictly comparable to the present method. In addition, the studies of Mugerwa and Conrad were performed on lactating dairy cows, which may have had a significant bearing on the divergency of results noted.

The present findings appear to indicate that during the summer growth phase, when the plane of nutrition is highest, reindeer and caribou probably recycle a proportion of the endogenous urea flux at least as great as the percent recycled by both cattle and sheep. In

terms of relative quantities of urea recycled per unit of metabolic body size, the sheep appeared to have some advantage over both caribou and cattle. The results of Ford (1969) and of Cocimano and Leng (1967) as compared by Ford and Milligan (1970), indicated that, within physiological limits, the quantity of urea recycled in sheep was proportional to plasma urea concentrations. The present results support this proposal, in that cattle, with an average plasma urea concentration of 8.5 mg/100 ml, averaged only 0.32 g urea recycled per unit metabolic body size. On the other hand, caribou with an average plasma urea concentration of 15.8 mg/100 ml, and sheep with an average of 21.8 mg urea/100 ml plasma, recycled 0.48 g and 0.53 g of urea per unit of metabolic body size respectively.

Studies by Weston and Hogan (1967) on sheep, Vercoe (1969) on cattle and Thornton (1970c) on cattle and sheep, all showed that on low nitrogen diets, the limit to the transfer of urea from the blood to the rumen fluid occurs at lower plasma urea concentrations and at slightly lower rumen fluid ammonia concentrations in cattle than in sheep. The present results further suggest that on low protein diets supplying similar amounts of nitrogen per unit metabolic body size, cattle maintain a lower plasma urea level than do either sheep or caribou. It is therefore logical to suppose that the cattle would recycle a lesser quantity of urea to the digestive tract per unit metabolic body size, even though the proportion of the urea flux recycled is comparable to sheep and caribou.

It is interesting to note that, although there is some controversy over the mechanism by which urea is transferred from the blood to the rumen fluid, several groups working with sheep have reported an

upper limit to this transfer at approximately the same range of plasma urea concentrations (Cocimano and Leng, 1967; Ford, 1969; Houpt and Houpt, 1968; Thornton, 1970c; Weston and Hogan, 1967). Thornton has suggested that the disparity reported between cattle and sheep with respect to this upper limit to urea transfer (Thornton, 1970c; Vercoe, 1969) "may have a physiological basis, such as differences in permeability to urea and/or the extent of urea degradation in various sections of the gut tract ... " It is possible that further disparities may exist with respect to other ruminant species.

Perhaps the most interesting results from Tables 4 and 5 concern the winter performance of the caribou bulls, both in terms of proportion of urea flux recycled and quantity of urea recycled per unit of metabolic body size. Although intake of nitrogen per unit of metabolic body size was reduced approximately one third from summer trials, the proportion of the urea of plasma that was recycled averaged 58%, and the quantity of urea recycled increased to 0.65 g per kilogram$^{3/4}$. Blood urea levels were slightly higher than the average summer levels, which was rather unexpected considering the drastic reduction in nitrogen intake. Under the limitations imposed by a simulated winter plane of nutrition, caribou appeared to gain some advantage over both sheep and cattle, and their own summer performance, in all aspects of urea recycling. The results presented in this study do not provide unequivocal statistical evidence for such a statement, but a trend definitely appears to exist.

In the statistical analysis presented in Table 5, a comparison between caribou in winter and summer is offered with the results from Trial III omitted. It is highly suspected that the results from

Trial III are errant, probably due to incomplete ^{14}C-urea injection into the jugular vein when the animal became over-excited. This would have resulted in a low recovery of label relative to the dose thought to have been injected, and therefore a high proportion would have been calculated to have been recycled. It is not possible to prove the injection was faulty. However, there was close agreement in plasma urea concentrations and urinary urea excretion between Trial III and all other summer trials. Furthermore, the high degree of uniformity between all other summer trials in the percent urea flux recycled, regardless of animal used, is strong evidence for the assumption. It is, however, possible that such variations in urea recycling can occur within one animal over a short period of time, and for this reason the results of Trial III are reported.

The extent to which the present results can be extrapolated to free-ranging caribou is speculative. The simulated winter ration reduced the total nitrogen intake signficantly from summer levels, but the major portion of the diet was still a balanced, 12% crude protein, complete ration. Lichen accounted for only 10-15% of the total nitrogen intake of the animals, although comprising from 35-45% of the diet by weight. Scotter (1967) has estimated that lichen accounts for 50-60% of the winter diet of feral caribou, but he has also suggested that caribou might have access to some forage of 5-10% protein content. The high protein content of the concentrate portion of the simulated diet, relative to natural forage, might tend to promote increased rumen ammonia concentrations, resulting in elevated plasma urea levels. However, a readily utilizable energy source is thought to inhibit such a situation by stimulating microbial synthesis (Packett and

Groves, 1965; Thornton, 1970c). A major benefit of the lichen portion of the simulated diet might be in the maintenance of rumen ammonia concentrations at a lower level, since reindeer and caribou digest the high crude fiber of lichen rather well (Nordfeldt et al., 1961). It is perhaps questionable if free-ranging animals in winter are able to gain access to the amount of crude protein given the experimental animals in this study. The blood urea levels of the experimental animals may have been higher than those existent in free-ranging caribou during the winter. A lower total crude protein intake could have important implications both in terms of amount of urea recycled, and the proportion of the urea flux recycled.

Realizing the limitations of the present studies, it nevertheless seems justifiable to suggest that caribou may have a greater potential to recycle urea than do typical domestic species of ruminants. It also seems probable that the full expression of this potential is realized only during the winter maintenance period; regulation is possibly associated with reproductive processes and the cyclical nature of the growth pattern.

A brief summarization of pertinent observations from this study leads one to speculate on the mechanisms by which urea recycling could be increased. Firstly, blood urea levels in the caribou in the winter were equal to, or slightly higher, than blood urea levels during the summer, although winter intake of nitrogen was significantly reduced on a metabolic body size basis. If the amount of urea recycled to the rumen is proportional to the plasma urea concentration, as suggested by the work of Ford (1969) and the present results, then the animal may gain a definite advantage by maintaining plasma urea at the

highest possible level. One seemingly obvious means of maintaining higher plasma urea concentrations would be through increased kidney conservation of urea. This control might be exerted at the kidney tubular level, possibly through regulation of a counter-current multiplier for concentrating urea in the urine (Schmidt-Nielsen, 1958), or the control might involve increased reabsorption of urea from the collecting ducts. The work of Vercoe (1969) suggested that there may be intra-species and inter-species differences in the ability to retain urea in the blood, due to differing capabilities, and regulation thereof, for concentrating urea in the urine and for reabsorbing urea from the urine.

Control of urea excretion at the kidney tubular level was not evident from the present results however, in that urea excretion per unit of metabolic body size was similar in both summer and winter and amounted to 0.46 g urea/kg$^{3/4}$ per day. Furthermore, urea excretion in the cattle was 0.35 g urea/kg$^{3/4}$ per day, which was lower than in the caribou, while, on the basis of plasma urea concentrations, one might have expected less conservation of urea by the kidneys of the beef bulls. No explanation for these phenomena appears readily available at this time, except that the amount of nitrogen available to the beef bulls may not have been as great as estimated, due to a low digestibility of the roughage fed.

Two possible means whereby the winter quantity of urea transferred from blood to the digestive tract could be increased over summer values can be envisaged. The plasma urea level could be substantially raised above the summer value, which apparently did not occur, or the plasma urea level could be maintained at the same level and the proportion of

the urea flux recycled increased. The experimental results obtained support the latter alternative, in that a trend toward a greater percent of label recycling did occur, in conjunction with an essentially equivalent plasma urea level. It is an intriguing possibility that the proportionality curve of plasma urea versus urea recycled, i.e. the disposition of urea, is shifted under winter conditions. Further experimental work, employing manipulation of blood urea levels under both summer and winter conditions, would be required to determine if a linear relationship between plasma urea concentration and quantity of urea recycled was maintained under winter conditions in caribou.

The mechanism(s) by which the proportion of plasma urea recycled to the digestive tract may be increased in caribou under conditions of low protein intake is not immediately evident. Changes in the permeability of the digestive tract toward urea may occur, although no evidence for, or against, such a supposition can be presented from this data. The theory which involves rumen ammonia concentration as a central factor in the control of urea transfer to the rumen may be relevant to the current findings.

The theory suggests that the amount of urea nitrogen transferred from the blood to the rumen is dependent on the blood urea level only as long as the rumen ammonia concentration remains sufficiently low (Varady et al., 1967). In addition, high dietary source of energy is thought to result in a low rumen fluid ammonia concentration by encouraging a more rapid rate of incorporation of ammonia into microbial protein (Packett and Groves, 1965; Thornton, 1970c). It is further suggested that a sufficiently low rumen fluid ammonia concentration allows the transfer of blood urea to the rumen in proportion to

the blood urea level. McEwan (1968a) and Scotter (1965) suggested that the winter feed intake of free-ranging caribou has a high energy to protein ratio, both in absolute terms and relative to the summer diet. This is due to the large proportion of lichen in the winter diet compared to almost no lichen in the summer diet (Klein, 1970). Lichen is known to have a high available energy content, especially for reindeer (Nordfeldt et al., 1961; Scotter, 1965). Furthermore there is evidence that the highly digestible nitrogen-free extract may increase slightly during the winter as the protein content tends to decrease slightly (Scotter, 1965). It therefore seems possible that the proportionality curve of plasma urea versus recycled urea is shifted toward a greater percent of the urea flux being recycled during winter, because a very high energy to protein ratio in the diet causes rumen ammonia concentrations to remain at lower levels than occur on summer forage. This could also mean a greater quantity of urea is recycled to the rumen in the winter. The recycled urea should be incorporated into microbial protein quite efficiently due to the ready availability of energy in the rumen. The work of Ford (1969) supports this hypothesis, in that he noted an increase from 32% of the urea flux recycled when an all barley diet was fed, to 46% recycled when roughage diets were fed to sheep. Since the simulated winter diet used in the present trials was from 35-45% lichen on a weight basis, it is suggested that the energy to protein ratio of the diet used could have approached that of a natural forage winter diet, especially if any significant amount of forage of 5-10% protein content is ingested by feral caribou (Scotter, 1967).

One further, very important point to be considered concerns the

partitioning of recycled blood urea between the direct transfer and salivary routes. No mention of salivary urea nitrogen has as yet been made in the text of this thesis, because no independent measurements of salivary urea were made, and the technique employed does not differentiate between the two modes of transfer of blood urea to the digestive tract. Although all discussion in this thesis generally alludes to direct transfer of blood urea across the rumen wall, it must be realized that in actual fact the data presented represents both modes of transfer. Indeed, further studies would have to be conducted to differentiate between the quantitative significance of the alternate means of transfer of plasma urea in _Rangifer tarandus_.

GENERAL CONCLUSIONS

The availability of animals suitable for experimentation dictated that the reported studies be performed on adult, male *Rangifer tarandus*. It is recognized, however, that the use of mature bulls may not provide the most informative data concerning urea recycling. It may have been preferable to have performed the trials on young growing animals, to compare the extent of urea recycling during the summer growth and winter dormancy phases of the first year of life. Since the summer growth rate of caribou calves in their first year is the greatest ever attained, the metabolic demands for nitrogen must be very high during this period. Even though growth ceases during the extreme environmental conditions endured during the first winter, and, in fact weight losses are common (McEwan, 1968a), it nevertheless seems logical that nitrogen conservation mechanisms might function to the maximum potential of the species and the individual. Future work utilizing growing animals might indicate that an even higher maximum potential to recycle urea exists in this species than was indicated here. In addition, the ratio of urea recycled in winter versus summer may prove to differ significantly when animals of varying sex and age characteristics are considered.

Comparative trials on breeding females are also considered important to a thorough investigation of this topic, since gestation over the winter period, in effect, constitutes a condition of growth in the female animal. Although body fat stores can be utilized to partially meet the increased energy demands associated with gestation, protein deficiencies of the winter diet would seem to make maximal nitrogen conservation imperative. In this regard, experiments

conducted by Nolan and Leng (1970) to compare the metabolism of urea in pregnant and non-pregnant sheep indicated that urea pool size, urea space, urea entry rate and rate of degradation of urea in the digestive tract, were all greater in pregnant than in non-pregnant sheep. Increases in these parameters were particularly pronounced under conditions of low nitrogen intake.

Trials designed to estimate the extent of urea recycling in lactating caribou females might also prove enlightening, since under feral conditions metabolic preparations for milk production may occur before the nutritive quality of the forage has improved to summer levels. Increased nitrogen requirements at this time associated with the production of milk of extremely high protein content (Tener, 1965), antler growth, and recovery of body tissues from winter stresses may be met through a superior ability to conserve and recycle blood urea.

The above speculations are intended to suggest that a great deal of additional investigation is required on urea recycling in *Rangifer tarandus*, in order to elucidate this topic with completeness.

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B30017